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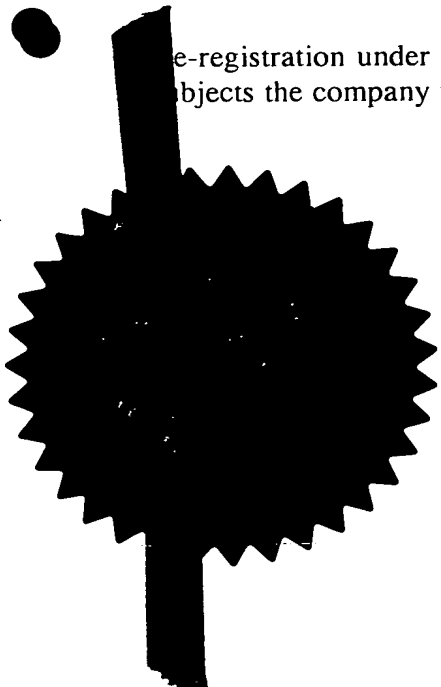
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*R. Mahoney*

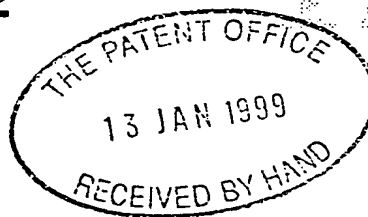
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Dated 17 November 1999



# Request for the grant of a patent

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The Patent Office

Cardiff Road  
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12 JAN 1999

1. Your reference

REP05997GB

2. Patent application number

(The Patent Office will fill in this part)

9900708.0

3. Full name, address and postcode of the or of each applicant (underline all surnames)

Microscience Ltd.  
67-68 Jermyn Street  
London  
SW1Y 6NY  
United Kingdom

Patents ADP number (if you know it)

If the applicant is a corporate body, give the country/state of its incorporation

United Kingdom

7583685001

4. Title of the invention

VIRULENCE GENE AND PROTEIN, AND  
THEIR USE

5. Name of your agent (if you have one)

GILL JENNINGS & EVERY

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

Broadgate House  
7 Eldon Street  
London  
EC2M 7LH

Patents ADP number (if you know it)

745002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

Country

Priority application number  
(if you know it)

Date of filing  
(day / month / year)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

Date of filing  
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8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if:

YES

a) any applicant named in part 3 is not an inventor  
b) there is an inventor who is not named as an applicant, or

c) any named applicant is a corporate body.

See note (d))

# Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form.  
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Continuation sheets of this form

Description

4 *SA*

Claim(s)

1

Abstract

Drawing(s)

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (*Patents Form 7/77*)

Request for preliminary examination and search (*Patents Form 9/77*)

Request for substantive examination (*Patents Form 10/77*)

Any other documents  
(*please specify*)

11. For the Applicant  
Gill Jennings & Every

I/We request the grant of a patent on the basis of this application.

Signature

Date

13 January 199

12. Name and daytime telephone number of person to contact in the United Kingdom

PERRY, Robert Edward  
0171 377 1377

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## VIRULENCE GENE AND PROTEIN, AND THEIR USE

### Field of the Invention

This invention relates to a virulence gene and protein, and their use. More particularly, it relates to  
5 their use in therapy and in screening for drugs.

### Background of the Invention

*E. coli* is an organism that is implicated in septicaemia, meningitis, urinary tract infection, wound infection, abscess formation, peritonitis and cholangitis.  
10 It would be desirable to provide means for treating or preventing conditions caused by *E. coli*, e.g. by immunisation.

In prokaryotes glycosyltransferases transfer activated sugars to a variety of substrates including  
15 glycogen, fructose-6-phosphate, lipopolysaccharide and capsular polysaccharide. Members of the group 1 glycosyltransferases transfer either UDP, ADP, GDP or CMP linked sugars.

### Summary of the Invention

The present invention is based on the discovery of a virulence gene in *E. coli* K1, which has been designated *eck1*, that encodes a protein with homology to several group  
1 glycosyltransferases from a number of bacteria.  
25 Accordingly, the present invention provides:

The therapeutic use of a peptide encoded by the operon including the *eck1* gene in *E. coli* K1, or a homologue thereof in a Gram-negative bacterium, or a functional fragment thereof, e.g. a peptide comprising all  
30 or part of the 150-member amino acid sequence defined below;

a host transformed to express the peptide or modified to disrupt expression of the gene;

a vaccine comprising such a peptide or the means for  
35 its expression, or an attenuated vaccine in which the virulence gene is disrupted;

the use of the peptide or corresponding polynucleotide as a target for screening potentially useful drugs, especially anti-microbials, or as a diagnostic agent in the detection of virulence, e.g. for testing for the presence of virulent coliforms in livestock.

#### Description of the Invention

The virulence gene in *E. coli* K1 was identified by using signature-tagged mutagenesis (STM) to screen an *E. coli* K1 mini-Tn5 mutant bank for attenuated mutants, in a mouse model of systemic infection. Bacteria containing a mini-Tn5 insertion within the virulence gene failed to be recovered from mice inoculated with a mixed population of mutants, and are therefore likely to be attenuated.

The cloned *E. coli* K1 nucleotide sequence following the mini-Tn5 insertion is as follows:

Length: 454

```
1  TGATTTTGA GATAATAACT GACAATCTCT AATTTCAAAC AAACAACCAT
20 51  TATAGCCATC TTCTATTAAG CTATTATTAC CTGGAATATT AGTGACTATA
    101 CATGGAAGTC CACAGCTCAA TGCTTCTAAA ATTGCTAATG GCATACCCTC
    25 151 CCAAAGAGAA GGTAATATAA AAAGATCATT AACTTTTAAA ATATTAACAA
    201 TGTTATCTGA CCATCCATGA AAAATTATAC GTCCATCTTG CCGTTTGAAC
    251 CTGCTTTCTA ACTGTTCTTT TAGTTCACCA TCTCCTACAA GTGTCAGCTT
    30 301 AACATTAACA TTTTCATTCA GCAGTTTTTC AACAGCAAGC AATAATGTCT
    351 CAGGATCTTT TTGCTTGGAT AATCTACCAA CCATTACTAG ATTCAAGGTG
    35 401 CTACTATAAA TTTTATTTTC TAAAGGAGAA AACTTATCAG TGTCTACTCC
    451 ATTA
```

A translation of this sequence is as follows:

Length: 150 amino acids

```
1  GVDTDKFSPL ENKIYSSTLN LVMVGRLSKQ KDPETLLLV EKLLNENVNV
    51  KLTLVGDGEL KEQLESRFKR QDGRIIFHW SDNIVNILKV NDLFILPSLW
    101  EGMPILAILEA LSCGLPCIVT NIPGNNSLIE DGYNGCLFEI RDCQLLSQKS
```

This amino acid sequence shows 37.5% identity to the *epsG* protein of *Streptococcus thermophilus*, (accession number Q56044) at amino acids 197-326 of the latter.

5 GCG bestfit analysis at the amino acid level is as follows.

```

197 MVGRLSPPKKEFFFFIDFAKKILQIRNDTNFIIVGDGELRSEIERMILDNG 246
    ||||| |: : .|:| : |||||: ::|
10 23 MVGRLSKQKDPETLLLAWEKLLNENVNVKLTlVGDGELKEQLESRF..KR 70
    247 LGDKIYITGWVDNPRNYIEKFDQAILFSRWEGLSLTIAEYMSQKKTILAT 296
        :| ||| | :. | || | ||: | | :| :|
15 71 QDGRIIFHGWSNIVNlLKVNDFILPSLWEGMPLAILEALSCGLPCIVT 120
    297 NIGGINDLITDGETGMLIEVGBLNSAVSKS 326
        || | | || | | | | : | ||
121 NIPGNNSLIEDGYNGCLFEIRDCQLLSQKS 150

```

20 The 150 amino acid sequence also shows 33% identity to a hypothetical protein from *Synechosystis* spp. (accession number P73948)

GCG bestfit analysis at the amino acid level is as follows

```

25 177 GVAVERYCPGQNDLKKEYQAERLFIYLGRIAPEKNVEALLKG.WKFSDMG 225
    || ::| :| | | : ||: :|. | || |
    1 GVDTDKFSPLEN...KIYSSTLNLVMVGRLSKQKDPETLLLAWEKLLNEN 47
30 226 PHCKLLMVGDGILKSTLQTHYGPENGVHWLGFVADELTRIQLLRAADAFI 275
    . || :||| || |.: : | .| : :||: ||
    48 VNVKLTlVGDGELKEQLESRFKRQDGRIIFHGWSNl..VNlLKVNDFI 95
35 276 LPSLVEGLSLSLLEAMACGTACVATDAGADGEVLENG 312
    |||| ||: |.:|||: || | : | . :|. |
    96 LPSLWEGMPLAILEALSCGLPCIVTNIPGNNSLIEDG 132

```

40 The 150 amino acid sequence also shows homology to proteins from a wide range of prokaryotes. These proteins are listed in the *glycos\_transf\_1* family in the PFAM database of multiple protein alignments (accession number PF00534). These proteins include the hypothetical *Klebsiella pneumoniae* protein YC07 (SwissProt accession number Q48453), the putative colanic acid biosynthesis glycotransferase WcaL from *E.coli* K12 (SwissProt accession number P71243) and the hypothetical *Bacillus subtilis*

protein YPJH (SwissProt accession number P42982).

The novel gene has been tested for attenuation of virulence, using mixed infections, in a murine model of systemic infection (Achtman et al., 1983, Infection and Immunity, vol 39, pages 315-335), and shown to be attenuated with a competitive index (CI) of 0.025 (mean CI from three mice).

The *E.coli* K1 *eck1* gene is likely to be useful both in generating attenuated vaccine strains and as a target for antimicrobials.

For the purposes of this invention, the appropriate degree of homology is typically at least 50%, preferably at least 60% or 70%, and more preferably at least 80% or 90% (at the amino acid or nucleotide level).

It is evident that *E. coli* K1 strains containing disruptions of the invention are attenuated. The products of the invention may be immunogenic. They are therefore useful in therapy, and more particularly as a prophylactic, in a vaccine.

The protein may be purified. It may be sequenced. The corresponding full-length gene can thus be identified.

It can thus be prepared by recombinant technology, by expression in a suitable host. Active fragments and homologues can be identified. Vaccine compositions, including attenuated vaccines, can be formulated, with carriers and adjuvants as necessary or desired, and used in therapy, to provide an effective immunisation against *E. coli*. In some cases, antibody may be used, for passive immunisation. All these procedures are known to those of ordinary skill in the art, and do not affect the nature of the invention that has been made.



# CLAIMS

1. A peptide encoded by the operon including the *eck1* gene *E. coli* K1, or a homologue thereof in a gram-negative bacterium, or a functional fragment thereof, for  
5 therapeutic use.
2. A peptide according to claim 1, comprising the 150-member amino acid sequence defined herein.
3. A polynucleotide encoding a peptide according to claim 1 or claim 2, for therapeutic use.
- 10 4. A host transformed to express a peptide according to claim 1 or claim 2.
5. A vaccine comprising a peptide according to claim 1 or claim 2, or the means for its expression.
6. A vaccine comprising a microorganism having a  
15 virulence gene deletion, wherein the gene encodes a peptide according to claim 1 or claim 2.
7. Use of a product according to any of claims 1 to 4, for screening potential drugs or for the detection of virulence.
- 20 8. Use of a product according to any of claims 1 to 4, for the manufacture of a medicament for use in the treatment or prevention of a condition associated with infection by *E. coli*.

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Form 23/77 : 9.11.99

AGENT : Gill Jennings & Every